ILL Document Delivery

REG-14501067

VAUZGE

NLM -- W1 ME961P (Gen): Film S01242; E-Journal w/ILL access

US PATENT AND TRADEMARK OFFICE
SCIENTIFIC AND TECHNICAL INFO CTR
107 S. WEST STREET, PMB 803
ALEXANDRIA, VA 22314

ATTN:
PHONE: 571-272-2517
FAX: 571-272-0230
E-MAIL: STIC-DOCS@uspto.gov

SUBMITTED: 2008-09-10 15:03:01
PRINTED: 2008-09-11 10:42:29
REQUEST NO.: REG-14501067
SENT VIA: DOCLINE
DOCLINE NO.: 25584121

REG Copy Journal

TITLE:	METABOLISM: CLINICAL AND EXPERIMENTAL
PUBLISHER/PLACE:	W.B. Saunders Philadelphia, PA :
VOLUME/ISSUE/PAGES:	1986 Jun;35(6):505-14 505-14
DATE:	1986
AUTHOR OF ARTICLE:	Lillioja S;Foley J;Bogardus C;Mott D;Howard BV
TITLE OF ARTICLE:	FREE FATTY ACID METABOLISM AND OBESITY IN MAN: IN
ISSN:	0026-0495
OTHER NUMBERS/LETTERS:	Unique ID.: 0375267 25584121 3713512
SOURCE:	PubMed
MAX COST:	$4.00
COPYRIGHT COMP.:	Guidelines
CALL NUMBER:	W1 ME961P (Gen): Film S01242; E-Journal w/ILL access
REQUESTER INFO:	666941
DELIVERY:	E-mail Post to Web: STIC-DOCS@uspto.gov
REPLY:	Mail:

KEEP THIS RECEIPT TO RECONCILE WITH BILLING STATEMENT
For problems or questions, contact NLM at http://wwwcf.nlm.nih.gov/ill/ill_web_form.cfm or phone 301-496-5511.
Include LIBID and request number.

NLM Collection Access Section, Bethesda, MD

Free Fatty Acid Metabolism and Obesity in Man: In Vivo in Vitro Comparisons

S. Lillioja, J. Foley, C. Bogardus, D. Mott, and B. V. Howard

We have examined the relationship of free fatty acid (FFA) turnover and lipid oxidation rates in vivo to the size of body triglyceride stores and compared these findings with the in vitro lipolytic rates of isolated abdominal fat cells. The studies were performed in 20 Pima Indian women 18 to 35 years of age, both lean and obese. FFA turnover rate was measured using a 1-14C-palmitate infusion, lipid oxidation rate by indirect calorimetry using a ventilated hood, body composition by underwater weighing with correction for residual lung volume, and fat cell lipolytic rates in vitro by published methods. Both FFA turnover and lipid oxidation rates, expressed per kg of body fat, decreased with increasing degree of obesity (as measured by percent body fat) ($r = -0.90$, and $r = -0.75$, $P \leq 0.0001$, respectively). In contrast, the rate of lipolysis determined in vitro, expressed per kg of fat, increased with increasing degree of obesity ($r = 0.58$, $P < 0.01$). A ratio of FFA turnover/lipolysis, which directly compares these in vivo and in vitro measurements, decreased significantly with increases in the degree of obesity ($r = -0.81$, $P \leq 0.0001$). Furthermore, there were no positive correlations between the measures of in vivo FFA metabolism and in vitro lipolysis when both were expressed per fat mass, per fat cell number, or per fat cell surface area. The in vivo data also demonstrated that lipid oxidation could only account for 50% of the FFA disappearance rate. While lipid oxidation rate adjusted to the metabolic size increased with increasing plasma FFA concentration ($r = 0.75$, $P < 0.0003$), the nonoxidative component of the FFA turnover failed to increase with increases in plasma FFA concentration ($P = 0.5$). We conclude that FFA is not available in vivo in proportion to the size of the triglyceride stores. The reason for this is not due to an inability of fat cells to release their stored triglyceride as assessed in vitro. Hence, in vitro measurements of fat cell lipolysis cannot be used to directly predict in vivo FFA metabolism. The large nonoxidative FFA disposal is likely to be important in the regulation of plasma FFA concentrations.

FREE FATTY ACID release from adipose tissue is the sole mechanism for the mobilization of fat cell triglyceride. Defective FFA release in obese subjects will result in the maintenance of large fat stores and the perpetuation of obesity. Excessive FFA release, on the other hand, might lead to increased lipoprotein formation[1,2] or a worsening of reduced glucose disposal.[3,4] An understanding, therefore, of the control of plasma FFA availability has important implications in health and disease. The study of factors regulating plasma FFA availability necessarily involves not only an assessment of in vivo FFA metabolism, but also an assessment of the metabolism of isolated fat cells, the major source of plasma FFA. In vivo FFA availability or use, as measured by FFA turnover and lipid oxidation rate, assessment of body composition to measure total fat mass, and in vitro measurements of lipolytic rates in isolated fat cells, have not previously been studied in the same subjects to investigate possible mechanisms of regulation of in vivo FFA metabolism.

The current studies, therefore, were designed to address the following questions: How is FFA availability or use related to the size of tissue triglyceride stores? Does the in vitro metabolism of isolated abdominal fat cells predict the in vivo FFA metabolism? We have compared measurements of fatty acid turnover using 1-^{14}C-palmitate,[5] indirect calorimetry using a ventilated hood system,[6] and in vitro assessment of fat cell physiology[7] in the same subjects.

MATERIALS AND METHODS

Subjects

Twenty Southwest American Indian (Pima) women were selected to represent a wide range of obesity. They were studied in the Phoenix Clinical Diabetes and Nutrition Section. They consumed a weight-maintaining diet (45% carbohydrate, 40% fat, 15% protein). All subjects were in good health as assessed by a medical history, physical examination, and routine hematological, biochemical, and urine tests. None had diabetes or impaired glucose tolerance.[8] Body composition was estimated by underwater weighing[9] with simultaneous determination of residual lung volume. Percent fat was calculated according to Keys and Brozek.[10] These results were used to calculate fat mass and fat free mass. All subjects gave informed consent, and the studies were approved by the ethical committees of the National Institutes of Health and the Indian Health Service, and by the Gila River Indian Community. Clinical data for the subjects are shown in Table 1.

This report is a companion to reference 4. The numbers assigned to subjects in table 1 are the same as those subjects were given in table 1 of reference 4.

Experimental Protocol

After at least two full days on the metabolic unit and after an overnight fast, each subject had subcutaneous abdominal adipose tissue removed from the lateral aspect of the hypogastrium, inferior to the umbilicus (McBurney's point). The skin was infiltrated with 2% xylocaine. An elliptical incision 4 cm in length was made, and a wedge-shaped sample of subcutaneous fat underlying the incision (5 to 15 g) was dissected.

After at least five days on the above diet and after an overnight fast of 13 to 14 hours, measurement of FFA turnover was performed with simultaneous indirect calorimetry. Fasting insulin levels reported are the means of duplicate samples taken on three separate days of the stay in the research unit.

In Vitro Experimental Methods

Isolated adipocytes were prepared by the collagenase method as described previously.[7]

From the Clinical Diabetes and Nutrition Section, National Institute of Arthritis, Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, Phoenix.

Address reprint requests to BV Howard, PhD, Clinical Diabetes and Nutrition Section, National Institute of Arthritis, Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, 4212 N 16th St, Room 541, Phoenix, AZ 85016.

0026-0495/86/3506-0006$03.00/0

Table 1. In Vivo and in Vitro Data of 20 Pima Indian Women

Subject	Weight (kg)	Height (cm)	Age (yr)	% Fat*	Fat Mass (kg)	Fat Free Mass (kg)	Average Cell Size (μg lipid/cell)	Cell Number†	FFA (μmol/L)	FFA Turnover (μmol/min)	Lipid Oxidation Rate (μmol/min)	Lipolysis (μmol/min)	Lip Coll‡	Fasting Insulin§	ED50 Antilipolysis‖
1	47.9	157	23	25	11.8	36.1	0.34	34	398	360	300	103	3.0	13	1.1
2	49.6	161	33	24	11.8	37.8	0.19	62	473	436	258	75	1.2	16	2.1
3	52.4	160	21	27	14.1	38.3	0.42	34	367	444	263	81	2.4	15	1.4
4	52.8	156	22	29	15.5	37.3	0.58	26	414	410	256	127	4.8	24	1.9
5	53.5	161	24	20	10.8	42.7	0.40	27	211	273	158	64	2.4	11	0.6
6	54.3	165	19	27	14.5	39.8	0.28	51	320	352	243	61	1.2	18	0.7
7	56.6	174	19	26	14.5	42.1	0.44	32	191	277	135	97	3.0	20	2.6
8	59.0	158	27	29	17.0	42.0	0.54	31	297	312	174	131	4.2	14	0.6
9	66.8	156	25	38	25.5	41.3	0.71	36	305	361	242	173	4.8	26	3.0
10	73.5	164	22	39	28.7	44.8	0.66	44	426	411	361	419	9.6	27	3.0
11	76.2	163	24	39	28.7	46.6	0.67	43	410	490	191	232	5.4	24	5.3
12	79.9	165	28	38	30.3	49.6	0.61	49	227	396	157	207	4.2	23	1.9
13	91.2	160	33	39	35.9	55.3	0.45	79	184	468	119	190	2.4	22	3.4
14	97.2	166	26	41	40.1	57.1	0.76	53	445	591	386	317	6.0	45	12.6
15	108.9	166	25	41	43.6	63.3	0.84	52	289	486	143	436	8.4	46	3.0
16	108.0	161	20	42	45.1	62.9	0.61	73	227	687	145	570	7.8	113	6.0
17	110.6	163	32	47	52.0	58.6	0.47	111	246	525	256	401	3.6	25	1.1
18	119.3	156	35	49	58.7	60.6	0.68	87	332	560	348	1299	15.0	49	3.7
19	63.6	160	31	32	20.1	43.5	0.55	36	223	381	155	153	4.2	59	2.0
20	67.1	164	26	32	21.4	45.7	0.61	35	441	521	189	106	3.0	37	1.7
Mean	74.3	162	26	34	27.0	47.3	0.54	50	321	437	224	262	4.8	31	3.0
± SE	± 5.2	± 1.0	± 1.1	± 1.8	± 3.3	± 2.0	± 0.04	± 5.0	± 21.2	± 23.8	± 17.9	± 63.6	± 0.74	± 5.2	± 0.6

*Determined by underwater weighing.

†Estimated fat cell number (10^9 cells).

‡Basal lipolysis per cell (μmol × 10^{-9}/min).

§Mean of duplicates on three mornings (μU/mL).

‖Insulin concentration for 50% suppression of lipolysis in vitro (μU/mL).

Adipose cell size was determined by sizing osmium fixed cells on a Coulter electronic counter with a 400-μm aperture[7] equipped with logarithmic range-expander channelyzer (Coulter Electronics, Irvine, Calif).[7]

Glucose incorporation into triglyceride was measured by incubating isolated adipocytes[8] in 1 mL Krebs-Ringer bicarbonate buffer equilibrated with 95% O_2 and 5% CO_2 (pH 7.4) containing 5% albumin, 0.5 μCi [U-^{14}C]-D-glucose and 5.5 mmol/L glucose and 8 nmol/L insulin at 37 °C for two hours with a continuous shaking at 40 cycles/min. The incubation was terminated by adding 300 μL of 8 sulfuric acid (normal concentration). Production of $^{14}CO_2$ was measured as described by Gliemann,[11] and glucose incorporation into triglyceride was determined by the method of Dole and Meinertz.[12]

Lipolysis was determined by incubating isolated adipocytes (4%) in 500 μL 5% albumin-Hepes buffer at 37 °C for two hours with continuous shaking at 40 cycles/min. The buffer contained either no isoproterenol or 25 nmol/L isoproterenol in the presence of 0, 12.5, 25, 50, 100, 200, and 8,000 pmol/L insulin. The incubation was terminated by the oil flotation method.[11] The incubation medium under the oil was taken for determination of glycerol. Glycerol was determined by an enzymatic assay essentially as described by Wieland.[13]

The concentration of insulin resulting in a half-maximum suppression of 25 nmol/L isoproterenol-stimulated lipolysis (ED50 antilipolysis) was calculated from equation of linear regression of percent lipolytic rate (+ insulin × 100/− insulin) *v* the log of the insulin concentrations at 12.5, 25, 50, 100, and 200 pmol/L. Since the maximum suppression of lipolysis occurred at 100 or 200 pmol/L insulin, the values at 100 or 200 pmol/L insulin were omitted if the values at these concentrations were at the maximum suppression of lipolysis. The correlation coefficient was greater than 0.95 in all experiments.

Chemicals. Collagenase (type 1) was obtained from Worthington (Freehold, NJ); albumin (bovine, fraction V) from Armour Pharmaceuticals (Kankakee, Ill); porcine insulin from Eli Lilly Company (Indianapolis); L-isoproterenol, L-glycine, ATP, β-NAD, glycerokinase, 9-glycerophosphate dehydrogenase, glycerol, and osmium tetroxide from Sigma (St Louis); hydrazine hydrate from Fisher Scientific Company (Fairlawn, NJ); silicone oil from Union Carbide Corporation (New York) [U-^{14}C]-D-glucose (329 mCi/mmol) from New England Nuclear (Boston), mono ^{125}I-(Tyr A 14)-insulin from NOVO, (Copenhagen); all other chemicals were of analytical grade.

In Vivo Experimental Methods

FFA turnover studies were performed using a modification of the method developed by Havel[5] using 1-^{14}C palmitate (New England Nuclear) as previously reported.[4] The fatty acid solution (10 mg/mL albumin, 0.5 μCi ^{14}C/mL, 2.3 mg palmitate/mL) was infused at a rate of 0.5 mL/min. Sixty-six minutes before the start of the insulin infusion, a plasma sample was obtained for baseline FFA concentration, and ^{14}C palmitate infusion commenced. Beginning 35 minutes after the ^{14}C palmitate infusion commenced, four blood samples were drawn over 23 minutes for determination of FFA concentration and specific activity.

Previous reports suggested the FFA specific activity would be at a steady state at the time of blood collection in the aforementioned protocol.[3,14,15] In this study the mean of the coefficients of variation (CV), calculated for each individual, of FFA specific activity was 7.7%. Specific activity showed no significant change over the study period (change = +2.9 ± 2.4%, P − 0.26).

During the 1-^{14}C palmitate infusion, oxygen consumption and carbon dioxide production were determined by open circuit indirect calorimetry.[6] A transparent plastic hood was placed over the subject's head and secured around the neck with a soft collar about 30 minutes prior to collecting the FFA samples for measurement of the FFA turnover. The flow rate was measured using a pneumotachograph attached to a Fleisch flow transducer (Gould, Cleveland). A constant fraction of the expired gases was withdrawn and analyzed for oxygen and CO_2 concentrations. The oxygen was measured by a zirconium cell analyzer and the CO_2 by an infrared analyzer (both from Applied Electrochemistry, Sunnyvale, Calif). The analyzers and flow meter outputs were connected to a desk top computer (Hewlett Packard, Palo Alto, Calif), which recorded continuous integrated calorimetric measurements over five-minute intervals. Nonprotein oxidation during the test was estimated from the urinary urea production rate. This was calculated as the urine urea concentration × urine volume/time since last voiding, on a specimen collected at the end of the euglycemic clamp, which followed the studies (see reference 4). The nonprotein respiratory quotient (NPRQ) was then calculated, and substrate oxidation values were determined from the equations of Lusk.[16]

Calculations

Substrate oxidation rates were calculated as previously described.[4] It should be noted that the constants used for lipid oxidation are those for the consumption of whole fat. Since this contains glycerol, the fatty acid oxidation (in mg) is overestimated. The difference would, however, be <4% if pure palmitate (RQ = 0.696), and <1% if pure oleate (RQ = 0.706) were assumed to be oxidized. Lipid oxidation in mg was converted to moles using the molecular weight of palmitic acid, so that the data for turnover and oxidation could be compared directly.

Rate of turnover of FFA was calculated assuming a steady state[5] as infusion rate (dpm/min)/specific activity (dpm/μmol). In this instance turnover rate = rate of appearance = rate of disappearance of FFAs.

Analytical Methods

Plasma glucose concentration was measured by the glucose oxidase method using a Beckman Glucose Analyzer (Fullerton, Calif). Plasma insulin concentrations were determined by the Herbert modification[17] of the radioimmunoassay of Berson and Yalow.[18] FFAs were measured by a modification[20] of the method of Soloni and Sardina.[19] The FFA assay was standardized by the inclusion of reference pools, which were calibrated by titration method of Dole[21] as modified by Trout.[22] All samples for fatty acid determinations were collected in tubes containing paraoxon (1.1 mg/2 mL) and kept on ice at all times.

Statistical Methods

Statistical calculations were performed using standard programs of the Statistical Analysis System (SAS) Institute, Cary, NC. Correlations are Pearson product-moment correlations. Log transformations of basal lipolysis, basal lipolysis/fat mass, ED50 for antilipolysis, fasting insulin, whole body nonoxidative FFA disposal, and turnover/kg of fat mass were used in the correlations to improve linearity and to decrease unequal variances.

RESULTS

Individual values for in vitro and in vivo measures of FFA metabolism and means for the group are shown in Table 1, and selected correlations in Tables 2 and 3.

In Vitro FFA Metabolism

With increases in degree of obesity (% fat) there were increases in the rate of basal lipolysis per adipose cell

Table 2. Relationship of % Body Fat and Fat Mass to Lipid Metabolism

	% Fat*		Fat Mass†	
	r	*P*	*r*	*P*
Turnover‡	.71	<0.0005	.75	≤0.0001
Turnover/fat mass§	−.90	≤0.0001	−.88	≤0.0001
Turnover/cell‖	−.34	NS¶	−.43	0.06
Turnover/surface area#	−.82	≤0.0001	−.82	≤0.0001
Lipid oxidation**	.21	NS	.16	NS
Lipid oxidation/fat mass††	−.75	≤0.0001	−.74	<0.0003
Lipid oxidation/cell‡‡	−.41	0.07	−.52	0.02
Lipid oxidation/surface area§§	−.70	<0.001	−.72	<0.0005
Lipolysis‖‖	.92	≤0.0001	.93	≤0.0001
Lipolysis/fat mass¶¶	.58	<0.01	.61	<0.005
Lipolysis/cell##	.72	<0.0005	.69	<0.001
Lipolysis/surface area***	.67	<0.002	.66	<0.002

*Determined by underwater weighing.
†% Fat × wt/100.
‡FFA turnover (μmol/min).
§Turnover/fat mass (μmol/min/kg).
‖Turnover/total body fat cell number (μmol/min).
¶Not significant ($P > 0.05$).
#Turnover/total body fat cell surface area (μmol/min/μm²).
**Lipid oxidation rate (μmol/min).
††Lipid oxidation/fat mass (μmol/min/kg).
‡‡Lipid oxidation/total body fat cell number (μmol/min).
§§Lipid oxidation/total body fat cell surface area (μmol/min/μm²).
‖‖Whole body lipolytic rate extrapolated from in vitro data (μmol/min).
¶¶Lipolysis/fat mass (μmol/min/kg).
##Basal fat cell lipolytic rate (μmol × 10^{-9}/min).
***Basal fat cell lipolytic rate/cell surface area (μmol × 10^{-16}/μm²/min).

(fmol/min) assessed in vitro ($r = .72$, $P < 0.0005$) (Fig 1). Basal lipolysis also increased significantly with average fat cell size (μg) ($r = .85$ $P \leq 0.0001$). The insulin concentration required for 50% suppression of isoproterenol simulated lipolysis (ED50-μU/mL) was significantly correlated with average cell size ($r = .59$ $P < 0.01$), and % fat ($r = .61$, $P < 0.005$).

Table 3. Metabolic Relationships of % Body Fat and FFA Concentretion

	% Fat*		FFA†	
	r	*P*	*r*	*P*
Turnover‡	.71	<0.0005	.21	NS§
Turnover/FFM‖	.07	NS	.72	<0.0005
Lipidox¶	.20	NS	.69	<0.001
Lipidox/FFM	−.23	NS	.75	<0.0003
Nonox#	.50	<0.03	−.28	NS
Nonox/FFM	.30	NS	−.17	NS
Lipidox/turnover	−.29	NS	.51	<0.03
Nonox/turnover	.29	NS	−.51	<0.03
Lipidox/RMR**	−.13	NS	.75	<0.0003

*Determined by underwater weighing.
†Plasma free fatty acid concentrations.
‡FFA turnover (μmol/min).
§Not significant ($P > 0.05$).
‖Fat free mass (wt − (% fat × wt/100)).
¶Lipid oxidation rate (μmol/min).
#Nonoxidative FFA uptake (turnover − lipidox, μmol/min).
**Resting metabolic rate (kcal/min).

In Vivo FFA Metabolism

As shown in Fig 2, increases in fat mass were not associated with equivalent increases in FFA turnover as indicated by the significant intercept on the y axis. Hence FFA turnover expressed per kg of fat mass significantly decreased with increases in fat mass ($r = -.88$, $P \leq 0.0001$). FFA turnover per kg of fat mass also decreased with increases in the degree of obesity expressed as % fat ($r =$

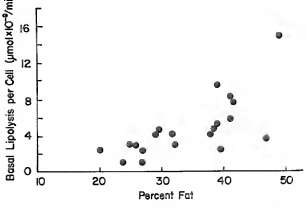

Fig 1. The relationship of basal lipolytic rate per cell measured in vitro to degree of obesity (% fat) ($r = .72$, $P < 0.0005$ for log lipolysis).

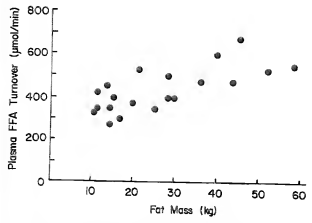

Fig 2. The relationship of whole body FFA turnover to fat mass ($r = .75$, $P \leq 0.0001$.) (intercept = 289, $P \leq 0.0001$.)

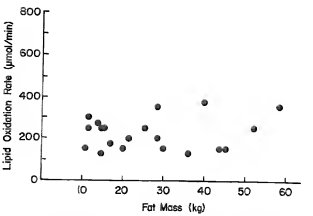

Fig 4. The relationship of lipid oxidation rate measured by indirect calorimetry in vivo to the fat mass. (NS, $P = 0.5$).

$-.90$, $P \leq 0.0001$) (Fig 3) since fat mass and % fat were themselves highly correlated ($r = .95$, $P \leq 0.0001$).

Increases in fat mass were not associated with increases in lipid oxidation rate (μmol/min, $P = 0.5$) (Fig 4). Similar to FFA turnover/fat mass, lipid oxidation rate per kg of fat mass was negatively correlated with degree of obesity ($r = -.75$, $P \leq 0.0001$). FFA turnover/fat mass and lipid oxidation/fat mass were also negatively correlated with average fat cell size ($r = -.68$, $P < 0.002$, $r = -.65$ $P < 0.002$). When FFA turnover or lipid oxidation were calculated per cell using the estimated total fat cell number, neither was correlated with % fat ($r = -.34$, $P = 0.14$; $r = -.41$, $P = 0.07$).

Larger fat cells have lower surface to volume ratios, resulting in decreased access of the triglyceride globule to the fat cell cytoplasm and cell surface. Hence, we estimated the total surface area of the fat cells per person. When either turnover or lipid oxidation was expressed per total body fat cell surface area, the negative relationships with % fat ($r = -.82$ $P \leq 0.0001$; $r = -.70$, $P < 0.001$) were similar to those found when the data were expressed per fat mass.

Using partial regression analysis, the relationship of FFA turnover/fat mass and lipid oxidation/fat mass with % fat persisted independently of the average cell size ($r = -.81$, $P \leq 0.0001$; $r = -.55$, $P < 0.02$).

Turnover per fat mass and lipid oxidation per fat mass also negatively correlated with the fasting plasma insulin ($r = -.52$, $P < 0.02$; $r = -.63$, $P < 0.01$) and weakly with the insulin concentration required for 50% suppression of lipolysis ($r = .42$, $P = 0.06$; $r = .43$, $P = 0.06$).

In Vivo in Vitro Comparisons

Since the basal lipolytic rate of isolated fat cells had initially been estimated per gram of fat tissue, an estimate of lipolysis for the whole body (whole body lipolysis) was calculated as (basal lipolysis per gram of fat cells) × (fat mass). This calculated value for whole body lipolysis is given in Table 1 and represented in Fig 5. In contrast to the in vivo measures of FFA turnover/fat mass and lipid oxidation/fat mass, whole body lipolysis/kg fat increased with % fat ($r = .58$, $P < 0.01$). When in vivo and in vitro measurements were equivalently expressed, there were no positive correlations between the in vitro and in vivo assessments of FFA metabo-

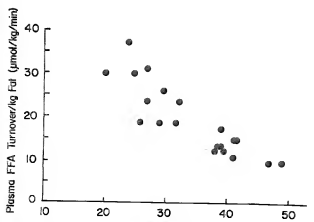

Fig 3. The relationship of plasma FFA turnover/kg of fat mass to the % fat (for log turnover/fat $r = -.90$, $P \leq 0.0001$).

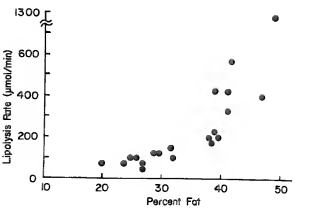

Fig 5. Relationship of whole body lipolytic rate (μmol/min) and the % fat, drawn on the same scale as Fig 6 ($r = .92$, $P \leq 0.0001$ for log lipolysis).

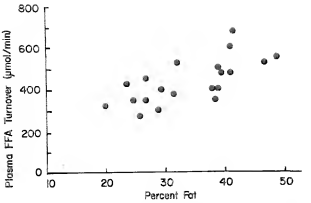

Fig 6. Relationship of whole body FFA turnover (μmol/min) and % body fat, drawn on the same scale as Fig 5 ($r = .71$, $P < 0.001$).

lism. The correlations are as follows: total body lipolysis/fat mass with FFA turnover/fat mass and lipid oxidation/fat mass ($r = -.51$ $P < 0.05$; $r = -.25$, $P = 0.3$); basal lipolysis/cell with FFA turnover/cell and lipid oxidation/cell ($r = .1$, $P = 0.7$; $r = 0$, $P = 1$); basal lipolysis/cell surface area with FFA turnover/cell surface and lipid oxidation/cell surface ($r = -.49$, $P < 0.05$; $r = -.30$, $P = 0.2$). The relationship of whole body lipolysis (lipolysis/g × fat mass) and % fat ($r = .92$, $P \leq 0.0001$ for log lipolysis) appeared to be nonlinear (Fig 5), in contrast to that of whole body FFA turnover (μmol/min) and % fat ($r = .71$, $P < 0.0005$) (Fig 6). To make a direct comparison between whole body FFA turnover determined in vivo and total lipolysis extrapolated from in vitro data, we calculated the ratio of FFA turnover/total lipolysis. This value was significantly negatively correlated with % fat ($r = -.81$, $P \leq 0.0001$) and is shown in Fig 7. Similarly, lipid oxidation/total lipolysis decreased with increasing % fat ($r = -.81$, $P \leq 0.0001$).

Nonoxidative FFA Disposal

In all subjects a significant amount of the FFA that disappeared from the plasma could not be accounted for by

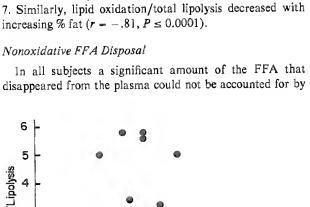

Fig 7. The relationship of the ratio of whole body FFA turnover/whole body lipolysis and the % fat ($r = -.81$, $P \leq 0.0001$).

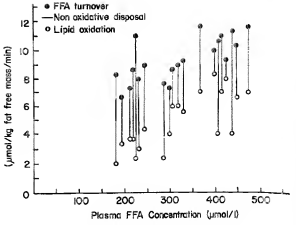

Fig 8. FFA turnover (●) plotted against the plasma FFA concentration ($r = .72$, $P < 0.0005$, slope = 0.012; intercept = 5.4, $P < 0.0001$ that it is different from zero) and lipid oxidation rate from indirect calorimetry (○) plotted against the FFA concentration ($r = .75$, $P < 0.0003$, slope = 0.015; intercept = 0.0, $P = 0$ that it is different from zero) nonoxidative FFA disposal shown as (|). (No correlation with plasma FFA $P = 0.6$). Slopes are not significantly different for the two regression lines ($P = 0.5$).

lipid oxidation (Table 1). The mean difference between turnover and lipid oxidation was 4.4 with a range 1 to 9 μmol/kg fat free mass (FFM)/min giving a ratio of lipid oxidation to turnover a mean of 53% (range 21% to 88%). Both FFA turnover and lipid oxidation expressed per kg of FFM correlated with the plasma FFA concentration ($r = .72$, $P < 0.0005$; $r = .75$, $P < 0.0003$) (Fig 8). The difference between these two, the nonoxidative disposal, did not correlate with FFA concentration ($P = 0.5$) or % fat ($P = 0.2$). Using partial regression analysis, FFA and nonoxidative disposal are significantly negatively correlated independent of the turnover/FFM ($r = -.68$, $P < 0.002$). This is a corrollary of the fact that lipid oxidation and FFA are positively correlated independent of the turnover rate/FFM ($r = .68$, $P < 0.002$). Nonoxidative disposal per kg of fat mass did not correlate with triglyceride synthesis/fat mass ($P = 0.08$), estimated in vitro as the amount of glucose incorporated into triglyceride.

DISCUSSION

The current studies were designed to assess the relationship of FFA metabolism and the body triglyceride stores and to determine if the size or physiology of isolated abdominal fat cells might predict the relationship between FFA metabolism and the fat depot. The results demonstrate that both FFA turnover and lipid oxidation rates, expressed per kg of fat mass, were lower in the most overweight subjects indicating that FFA availability or use is not in direct proportion to triglyceride store. That the size of the fat depots has some influence on the FFA availability, though not on oxidation, is suggested by the relationship of these two measures and the fat mass. The in vitro basal lipolysis rates, determined in isolated abdominal fat cells and extrapolated to the whole fat mass (μmol/min) appeared to increase exponentially with the degree of obesity. The whole body FFA turnover (μmol/

min), in contrast, showed much smaller increases with increases in % fat. A ratio of FFA turnover/whole body lipolysis, which directly compares the in vivo and in vitro measurements, significantly decreased with increases in % fat. Thus, we were not able to demonstrate any positive correlations between equivalently expressed in vivo and in vitro data. The results also showed that there was a large nonoxidative component of the FFA turnover. Subjects with the highest plasma FFA concentrations had the highest rates of lipid oxidation. This relationship persisted even when the turnover rate was taken into account using partial regression analysis. It is likely that this is a cause and effect relationship (see reference 4). The nonoxidative disposal, however, showed no such increase with plasma FFA concentrations. Indeed, partial regression analysis indicated that for any given turnover (rate of appearance), those subjects with the lowest nonoxidative FFA disposal had the highest plasma FFA. This is the corollary of the relationship of lipid oxidation and FFA. Given this evidence it seems possible that low non-oxidative FFA disposal rates would directly reduce FFA clearance and hence increase plasma FFA concentration.

In Vivo Studies

There are two possible mechanisms that might explain the decrease of turnover and lipid oxidation expressed per kg of fat mass with increasing obesity. The first of these possibilities is suggested by the fact that the relationship in Fig 2 is linear with a significant intercept, and perhaps by the high nonoxidative FFA disposal. These suggest that the fat mass may not be the only regulator of FFA supply. Alternatively, since in Fig 2 there are no data points less than 10 kg, it is possible that the turnover rate may become very small as the fat mass approaches zero. In this case the relationship of turnover to fat mass would actually be curvilinear, approaching an asymptote at high fat masses. In this situation the fat cell, or the fat cell environment, changes so dramatically with increases in obesity that the FFA stores are not readily available to the rest of the body.

In Fig 2 the significant intercept of turnover with the fat mass could imply that much of the FFA turnover occurs independently of the major fat stores in the adipose tissue fat mass. Skeletal muscle is known to contain significant quantities of triglyceride fatty acid in addition to phospholipid fatty acid. Denton and Randle[23] reported the triglyceride content of rat muscle (free of adipose cells) to be 12.0 umol of glyceride glycerol/g of dry muscle. If human muscle is comparable, then the total fatty acid content of skeletal muscle alone, and not including other nonadipose tissues, might range from 0.1 to 0.2 mol from our lean to obese subjects. This is much smaller than the 42 to 230 mol of total body triglyceride in these subjects but is greater than the hourly FFA turnover of 0.02 to 0.04 mol in the same subjects. This tissue FFA/triglyceride is likely to be a source for tissue lipid oxidation.[24] These tissues pools do not appear to be homogeneous, however, since lipid for oxidation can be derived either from pools that equilibrate with plasma FFA or from pools that do not equilibrate so readily.[25] Evidence for potential high rates of lipolysis in striated muscle is demonstrated by the fact that per unit weight, the rate of lipolysis in perfused heart muscle is as great as that of epididymal adipose tissue.[26,27]

There is evidence that FFA does appear in the plasma from nonadipose tissue. Labelled FFA from bolus injections is found predominantly in liver and skeletal muscle.[28] This FFA is presumably in the same FFA/triglyceride pools that FFA have been shown to enter prior to oxidation in skeletal muscle.[24,29] Other studies on the other hand have shown that 20% to 30% of injected labelled FFA in humans leaves the plasma and rapidly returns.[30] This FFA cycling has also been noted in other studies.[5,28,31] This may not be simply plasma/adipose tissue FFA cycling since only relatively small amounts of plasma FFA are taken up into this tissue.[28] The reduction of stainable lipid droplets in skeletal muscle in dogs within hours of stopping the excessive tissue accumulation of lipid brought about by norepinephrine infusions[32] would be consistent with a rapid exchange of nonadipose tissue triglyceride and plasma FFA.

These data then at least allow the possibility that some of the FFA appearance rate could be due to FFA that is leaving nonadipose tissue pools that have not reached equilibrium with the plasma labelled FFA.

Using indirect calorimetry we could measure the total rate of lipid oxidation. This was only about 50% of the total FFA turnover so that there was a signficant nonoxidative FFA disposal. Since lipoprotein synthesis can only account for a small fraction of the nonoxidative disposal,[1,2,33] the major part of the nonoxidative disposal could be net transfer of FFA from adipose tissue to other tissues, perhaps to replace FFA used during exercise[29] for example. Muscle can even accumulate triglyceride if FFA mobilization is excessive[32] and heart muscle actually accumulates triglyceride during prolonged fasting.[32] Using the rough calculations above, the nonoxidative disposal averages 8% of the muscle tissue stores per hour ranging from 2% to 16%. It is possible that in some subjects such rates of lipid storage could be excessive for nonadipose tissues under normal conditions. An alternate explanation, therefore, for the nonoxidative disposal could be a recycling of FFA from fat free tissue back to fat free tissue. Adipose tissue presumably does not directly participate in this cycle since it does not take up significant amounts of plasma FFA.

We conclude that Fig 2 raises the possibility that some of the FFA turnover occurs independently of the fat mass. To what degree it occurs is uncertain though some possible mechanisms exist. Figure 4 indicates that the rate of lipid oxidation may be entirely independent of the fat mass.

The second possible explanation for the lower FFA turnover or lipid oxidation per kg of fat in more obese subjects may be that there is some quality of adipose tissue, or its hormonal environment, that reduces the efficiency of FFA availability in obese subjects. The current data showed that the decreased surface to volume ratio of larger fat cells was not the reason for the decrease of turnover/fat mass or lipid oxidation/fat mass with increases in % fat. However, the estimation of whole body fat cell number cannot be regarded as any more than an approximation, since only fat cells of the abdominal depot were used. Any normalization of data to the fat cell number, therefore, is associated with considerable error. It is possible that the higher fasting insulin level in

some subjects could result in lower FFA release. Since fasting insulin level and ED50 for antilipolysis are correlated ($r = .67$, $P < 0.002$), higher insulin levels are likely to be offset by the greater fat cell resistance to insulin at these higher insulin levels. It is unclear, therefore, in these resting studies what role insulin may be playing in the lower turnover/fat mass or lipid oxidation/fat mass in some individuals.

Other adipose tissue factors, not measured in this study that are likely to influence FFA release, include plasma catecholamines, fat cell sensitivity to catecholamines, sympathetic nervous system activity, and capillary blood supply.

Finally, it is possible in vivo that both qualities of adipose tissue and its milieu and the handling of FFA by nonadipose tissue could be contributing to the observed FFA turnover and lipid oxidation. Most importantly, the data suggest that in obese subjects much of the increased fat stores may not be readily accessible to the rest of the body. Furthermore, the contribution of lipid oxidation to the overall metabolic rate was no higher in obese subjects ($P = 0.6$, Table 3) indicating a failure of obese subjects to make use of the increased stores of fat.

In Vivo in Vitro Comparisons

We compared the in vivo and in vitro measurements of FFA metabolism in order to see if the changes in in vivo FFA metabolism with obesity might be explained by the metabolism of isolated fat cells. Basal lipolysis in vitro had initially been measured per gram of fat cells, and it seemed reasonable, therefore, to multiply this by the fat mass to predict a whole body lipolysis rate. Basal lipolysis was assumed to be generally representative of the fat cells' ability to release triglyceride. Basal lipolysis can be stimulated by catecholamines, but only under these conditions is lipolysis suppressed by insulin.

The differences between the in vivo and in vitro measures of FFA metabolism are illustrated in Figs 5 and 6. We have suggested earlier that the nonadipose tissue might contribute to the FFA turnover. If this were the case, then those subjects with the lowest % fat (ie highest % lean) might have the greatest relative effect of FFA metabolism in skeletal muscle or liver. The data in Figs 5 and 6 suggest, however, that a major difference between the in vivo and in vitro measures of FFA metabolism occur in obese subjects in whom the lipolysis extrapolated to the whole body increased steeply with increases in % fat whereas the in vivo measures did not. It is possible that obese subjects have a very high capacity for lipolysis but that plasma or local tissue factors alter this FFA supply.

The ED50 was approximately 7% (range 3% to 28%) of the fasting insulin concentrations. Since these fat cells were maximally stimulated with isoproterenol, the difference between the fasting insulin concentration in vivo and that concentration required for 50% suppression of lipolysis in vitro is even more striking. These data suggest that the in vivo fat cell hormonal environment might be quite different from that indicated by the plasma hormone levels.

We suggest, therefore, that the adipose cell environment in vivo, particularly its distance from capillaries, which can lead to gradients of FFA from cell to plasma and gradients of hormones from plasma to cell, will limit the use of in vitro measurements of fat cell lipolysis in directly predicting in vivo fatty acid metabolism.

Nonoxidative FFA Disposal

In all subjects the total FFA turnover greatly exceeded the lipid oxidation, as observed by others.[33-36] In Fig 8 both the lipid oxidation and FFA turnover rates increased with increasing FFA concentration. The latter, however, has a significant intercept on the y axis. A calculation of FFA clearance from this data (ie turnover/FFA concentration) results in lower values with higher FFA concentrations. This fall in clearance with higher FFA has been reported by others.[31] This can be contrasted with the data of Galster et al.[37] The relationship of turnover of FFA to plasma FFA in the resting state is similar to that in our data. When FFA levels were raised however, with norepinephrine, the FFA clearance was either constant or it increased. The simple calculation of turnover/FFA concentration in Fig 8 demonstrates that this is not what would be predicted from the group regression in the resting state in our data (or theirs). Since in our data, the nonoxidative FFA disposal did not increase with increases in plasma FFA levels, we speculate that the plasma FFA levels produced by a given FFA appearance rate (turnover) are much higher than they would have been. This speculation is supported by the partial regression analysis, which demonstrated a significant negative correlation of FFA and nonoxidative disposal independent of the turnover rate (ie, for any given rate of FFA appearance, the highest FFA concentration was in the subjects with the lowest nonoxidative disposal). Figure 9 is a

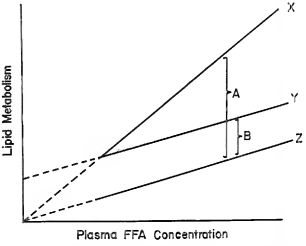

Fig 9. Actual relationship of lipid oxidation rate to plasma FFA concentration (line Z). Actual relationship of turnover to plasma FFA concentration (Line Y) (clearance decreases with increases in FFA concentration). Relationship of turnover to plasma FFA if clearance does not decrease with increases in plasma FFA (Line X). Actual nonoxidative metabolism (Y-Z) is represented by (B). Nonoxidative metabolism for constant value of FFA clearance as plasma FFA and turnover increase (X-Z) represented by (A). It can be seen that at a given turnover plasma FFA concentration is considerably higher in the absence of an increased nonoxidative disposal.

theoretical representation of the relationships of FFA turnover and plasma FFA concentration, which explains why subjects with higher plasma FFA have lower clearance of FFA while raising FFA with norepinephrine infusions is not associated with a reduced FFA clearance. These data suggest a critical role for the nonoxidative FFA disposal in regulating FFA concentrations. As discussed earlier this nonoxidative process is likely to be reesterification of fatty acid particularly in skeletal muscle or liver either as net transport of FFA from adipose tissue to these tissues or as a substrate cycle of plasma FFA/tissue triglyceride.

A possible regulatory mechanism for this nonoxidative disposal is suggested by studies during total parenterral nutrition.[34] In these studies increasing carbohydrate loads reduced lipid oxidation more than turnover (ie, nonoxidative disposal increased). Hence, the supply of glucose might have encouraged triglyceride synthesis by supplying alpha-glycerol phosphate.[38,39] Improved tissue supply of glucose might also explain why the treatment of diabetes appears to improve FFA clearance more than reducing FFA turnover.[36]

In summary, the significant nonoxidative FFA metabolism demonstrated in these and other studies is likely to play an important role in regulating plasma FFA concentrations. What the mechanisms for the control of this process might be are not fully known at this time.

CONCLUSION

The data indicate that fatty acid availability or use per kg of fat actually decreases with increasing obesity. This could be due to a contribution of nonadipose tissue triglyceride to the FFA turnover and lipid oxidation. Even more likely, however, is that plasma or local tissue factors in obese individuals prevent fatty acid from becoming available in spite of increased stores. This was also suggested by the findings in vitro. These data suggested that the fat cells were more than capable of releasing large amounts of FFA in obese subjects but apparently failed to do so in vivo. These results indicate that in obese subjects much of the increased fat store may not be accessible to the rest of the body. Furthermore, in vitro measurements of fat cell lipolysis cannot be used to directly predict in vivo fatty acid metabolism. Finally, these data indicate that there is a large nonoxidative fatty acid disposal that may be important in the regulation of the plasma FFA concentration.

The current study was performed in Pima Indian women. As a racial group they are characterized by several metabolic abnormalities, particularly a high prevalence of obesity and diabetes mellitus. Whether the current data is applicable to caucasian women is currently under investigation. We have no data on FFA turnover available in males.

ACKNOWLEDGMENT

The authors thank Dr Peter Bennett and Dr William Knowler and other staff of the Epidemiology and Clinical Research Branch in Phoenix; James Smart, Nordisk, Bethesda, Md for the kind donation of the purified pork insulin; Marjorie Robinson and the nursing and dietary staff of the Clinical Diabetes and Nutrition Section; Verna Kuwanhoyioma, Marilyn Francisco, and Christa Byrd for secretarial assistance; Annette Kennedy, Tom Anderson, John Brown, Inge Harper, Victoria Mongillo, Suzzane Moser, Harlan Osife, Vera Rodriquez, Karen Stone, Pamela Thuillez, and Debbie Wolf-Lopez for technical assistance.

REFERENCES

1. Havel RJ, Kane JP, Balasse EO, et al: Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridaemic and hypertriglyceridemic humans. J Clin Invest 49:2017–2035, 1970

2. Howard BV, Zech L, Davis M, et al: Studies of very low density lipoprotein triglyceride metabolism in an obese population with low plasma lipids, lack of influence of body weight or plasma insulin. J Lipid Res 21:1032–1041, 1980

3. Randle PJ, Garland PB, Hales CN, et al: The glucose fatty acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785–789, 1963

4. Lillioja S, Bogardus C, Mott DM, et al: Relationship between insulin mediated glucose disposal and lipid metabolism in man. J Clin Invest 75:1106–1115, 1985

5. Havel RJ, Naimark A, Borchgrevink CF: Turnover rate and oxidation of free fatty acids of blood plasma in man during exercise: Studies during continuous infusion of palmitate-1-C14. J Clin Invest 42:1054–1063, 1963

6. Jequier E: Long term measurement of energy expenditure man: Direct or indirect calorimetry?, in Bjorntorp P, Cairella M, Howard AN (eds): Recent Advances In Obesity Research, vol 3. London, Libby, 1981, pp 130–135

7. Kashiwagi A, Verso MA, Andrews J, et al: In vitro insulin resistance of human adipocytes isolated from subjects with non insulin dependent diabetes mellitus J Clin Invest 72:1246–1254, 1983

8. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039–1057, 1979

9. Goldman RF, Buskirk ER: A method for underwater weighing and the determination of body density. in Brozek J, Herschel A (eds): Techniques for Measuring Body Composition. Washington DC, National Academy of Sciences, National Research Council, 1961, pp 78–106

10. Keys A, Brozek J: Body fat in adult man. Physiol Rev 33:245–325, 1953

11. Gliemann J: Assay of insulin-like activity by the isolated fat cell method. 1. Factors influencing the response to crystalline insulin. Diabetologia 3:382–388, 1967

12. Dole VP, Meinertz H: Microdetermination of long chain fatty acids in plasma and tissues. J Biol Chem 235:2595–2599, 1960

13. Weiland O: Glycerol (UV method), in Bergmeyer HU (ed): Method of Enzymatic Analysis. Orlando, Fla, Academic, 1974, pp 1404–1408

14. Hagenfeldt L, Wennlund A, Felig P, et al: Turnover and splanchnic metabolism of free acids in hyperthyroid patients. J Clin Invest 67:1672–1677, 1981

15. Friedberg SJ, Klein RF, Trout DL, et al: The characteristics of the peripheral transport of C14 labelled palmitic acid. J Clin Invest 39:1511–1515, 1960

16. Lusk G: Animal calorimetry: Analysis of the oxidation of mixtures of carbohydrate and fat. J Biol Chem 59:41–42, 1924

17. Herbert V, Lau K, Gottleib CW, et al: Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375–1384, 1965

18. Yalow RS, Berson SA: Immunoassay of endogenous plasma insulin in man. J Clin Invest 39:1157–1167, 1960

19. Soloni FG, Sardina LC: Calorimetric microdetermination of free fatty acids. Clin Chem 19:419–424, 1973

20. Howard BV, Savage PJ, Nagulesparan M, et al: Evidence for marked sensitivity to the antilipolytic action of insulin in obese maturity-onset diabetics. Metabolism. 28:744–750, 1979

21. Dole VP: A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J Clin Invest 35:150–154, 1956

22. Trout DL, Estes EJ Jr, Friedberg SJ: Titration of free fatty acids of plasma: A study of current methods and a new modification. J Lipid Res 1:199–202, 1960

23. Denton RM, Randle PJ: Concentration of glycerides and phospholipids in rat heart and gastrocnemius muscles. Biochem J 104:416–422, 1967

24. Dagenais GR, Tancredi RG, Zierler KL: Free fatty acid oxidation by forearm muscle at rest, and evidence for an intramuscular lipid pool in the human forearm. J Clin Invest 58:421–431, 1976

25. Robin AP, Nordenstrom J, Askanazi J, et al: Influence of parenteral carbohydrate on fat oxidation in surgical patients. Surgery 95:608–618, 1984

26. Denton RM, Randle PJ: Measurement of flow of carbon atoms from glucose and glycogen glucose to glyceride glycerol and glycerol in rat heart and epididymal adipose tissue. Biochem J 104:423–434, 1967

27. Garland PB, Randle PJ: Regulation of glucose uptake by muscle. Biochem J 93:678–687, 1964

28. Bragdon JH, Gordon RS: Tissue distribution of C14 after the intravenous injection of labelled chylomicrons and unesterified fatty acids in the rat. J Clin Invest 37:574–578, 1958

29. Issekutz B Jr, Miller HI, Paul P, et al: Source of fat oxidation in exercising dogs. Am J Physiol 207:583–589, 1964

30. Eaton RP, Berman M, Steinberg D: Kinetic studies of plasma free fatty acid and triglyceride metabolism in man. J Clin Invest 48:1560–1579, 1969

31. Fredrickson DS, Gordon RS, Ono K, et al: The metabolism of albumin-bound C14-labelled unesterified fatty acids in normal human subjects. J Clin Invest 37:1504–1515, 1958

32. Carlson LA, Liljedahl SO, Wirsen C: Blood and tissue changes in the dog during and after excessive free fatty acid mobilization. Acta Med Scand 178:81–102, 1965

33. Miller HI, Bortz WM, Durham BC: The rate of appearance of FFA in plasma triglyceride of normal and obese subjects. Metabolism 17:515–521, 1968

34. Nordenstrom J, Carpentier YA, Askanazi J, et al: Free fatty acid mobilization and oxidation during total parenteral nutrition in trauma and infection. Ann Surg 198:725–735, 1983

35. Owen OE, Trapp VE, Reichard GA Jr, et al: Nature and quantity of fuels consumed in patients with alcoholic cirrhosis. J Clin Invest 72:1821–1832, 1983

36. Taskinen MR, Bogardus C, Kennedy A, et al: Multiple disturbances of free fatty acid metabolism in non-insulin dependent diabetes: Effect of oral hypolycaemic therapy. J Clin Invest 76:637–644, 1985

37. Galster AD, Clutter WE, Cryer PE, et al. Epinephrine plasma threshold for lipolytic effects in man. J Clin Invest 67:1729–1738, 1981

38. Tzur R, Tal E, Shapiro B: Alpha-glycerophosphate as regulatory factor in fatty acid esterification. Biochim Biophys Acta 84:18–23, 1964

39. Shulman GI, Williams PE, Liljenquist JE, et al: Effect of hyperglycaemia independent of changes in insulin or glucagon on lipolysis in the conscious dog. Metabolism 29:317–320, 1980